A clinical analysis of clinicopathological features and prognostic factors of triple-negative breast cancer

By Oana-Adriana Rajput-Anghel

WORD COUNT	4395	TIME SUBMITTED	28-MAR-2024 03:28PM
		PAPER ID	107899458

A clinical analysis of clinicopathological features and prognostic factors of triple-negative breast cancer

Oana-Adriana Rajput-Anghel[1] Traean Burcos[1,2]

[1]Coltea clinical Hospital, Department of General Surgery, Bucharest, Romania

[2]Carol Davila University, Department of General Surgery, Bucharest, Romania

Abstract

Objectives. The aim of this study was to explore the clinicopathological characteristics, prognostic factors, recurrence patterns and survival analysis of triple negative breast cancer patients compared to non-triple negative breast cancer patients.

Materials and Methods. The cohort included 420 patients who were diagnosed with breast cancer. The patients were evaluated based on the molecular classification and grouped into TNBC and non-TNBC. Data was explored using SPSS Version 29.0.0.0. Patient and tumor characteristics were studied. Univariate and multivariate Cox Regression was used to analyse prognostic factors. Kaplan Meier method with the log-rank test was performed to observe DFS and OS.

Outcomes. The triple negative subtype was observed in 57(13.6%) patients. Patients with TNBC had a greater proportion of grade 3 tumors compared to those with non-TNBC (43.9% vs. 5.5%, p<.001). In the univariate analysis pathological type, tumor size, tumor grade, nodal invasion, lymphovascular invasion, perineural invasion and extra-nodal extension were identified as statistically significant prognostic factors. The mean time to relapse for TNBC was lower 60.0 (95% CI,

[1] Corresponding author: Oana-Adriana Rajput-Anghel
Telephone number: +40723935843
E-mail: dr.adrianaanghel@yahoo.ro

37.16 to 82.83) compared to the non-TNBC group with a mean time to relapse of 72.0 months (95% CI, 55.81 to 88.18), nevertheless distributions were not statistically significant $X^2(1)=1.524$, p.217. Mean overall survival was 80.53 months (95% CI, 77.75 to 83.32) in non-TNBC compared to TNBC of 80.04 month (95% CI, 66.76 to 93.33) with a non-statistically significantly Log rank test $X^2(1)=1.252$, p.263. **Conclusions.** Triple negative breast cancer represents a heterogenous molecular and prognostic group of tumors which needs further clinical trials in order to develop targeted therapies.

Keywords*:* breast cancer, triple negative, metastasis, prognostic analysis

Abbreviations: TNBC, triple negative breast cancer; HR, hormone receptors; ER, estrogen receptor; PR, progesteron receptor; HER2, human epidermal growth factor receptor 2; CNB, core needle biopsy; IBC-NST, invasive breast cancer of no special type; LN, lymph node; LVI, lymphovascular invasion; PNI, perineural invasion; ENE, extra-nodal extension; DFS, disease free survival, OS overall survival.

Introduction

Triple negative breast cancer (TNBC) defined by the absence of expression of ER, PR and HER2 is considered a heterogenous disease at the molecular, pathologic and clinical level representing for 15% to 20% of breast cancers [1,2]. In order to better identify molecular-based therapy, cluster analysis, according to gene expressions (GE) profiles, identified and developed four tumour molecular subtypes, respective basal-like 1(BL1), basal-like 2(BL2), mesenchymal (M) and luminal AR (LAR) [3-5]. Reviews regarding morphological features revealed higher grade tumors such as grade 3 IBC-NST, metaplastic carcinoma, adenoid cistic carcinoma, elevated mitotic count, geographic necrosis, a pushing border of invasion and a stromal lymphocytic response [6,7] also BRCA1 mutation beeing more frequently associated with this morphology [8].

The aim of this study was to identify the clinicopathological features of this breast cancer molecular subtype and analyse the prognostic factors in terms of survival.

Material and Methods

A total of 640 patients with the diagnosis of invasive breast cancer were registered in the Department of Surgery of Coltea Clinical Hospital in Bucharest between January 1, 2015 and 31 December, 2019. The patients who were diagnosed by core needle biopsy (CNB) and confirmed by surgery and pathological examination were retrospectively analyzed. The study protocol was approved by the Ethics Committee of the Coltea Clinical Hospital. For the study were eligible 420 patients and included patients whose immunohistochemical assessment of biomarkers and histopathology characteristics, in accordance with the classification of tumors developed by the World Health Organisation, were available. The patients were then grouped on the basis of expression of hormone receptors (HR) and HER2 in triple negative breast cancer (TNBC) and non-triple negative breast cancer (non-TNBC). TNBC was defined by the lack of estrogen receptors (ER), progesteron receptors (PR) and HER2 expression, confirmed by IHC and fluorescence in situ hybridisation (FISH). The further collected baseline data included age, menopausal status, tumor stage (based on TNM staging of breast cancer developed by the American Joint Committee on Cancer Staging Manual 8th Edition), pathological type, tumor size and grade, nodal invasion, lymphovascular invasion (LVI), perineural invasion (PNI), extra-nodal invasion (ENE)(defined as extracapsular extension of nodal metastasis) and recurrence.

Preoperative systemic therapy and systemic adjuvant treatment were administered based on on the recommendations of the National Comprehensive Cancer Network Guidelines for Breast Cancer. Neoadjuvant therapy was given to 191(45.5%) out of the 420 cases before surgery. A number of 340(81%) patients underwent Madden modified radical mastectomy (MRM), 62(14.8%) breast-conserving surgery (BCS) and 17(4.1%) toilet mastectomy. The final follow-up of all patients was completed in May 2022. Disease-free survival (DFS) was defined as the period from the first day after surgery to first locoregional recurrence or distant metastasis. Overall survival (OS) was defined as the period from the first day after surgery to death from breast cancer. The patients who did not experience any event or were lost to follow-up were censored for survival analysis.

To analyse the collected data was used statistical software IBM SPSS Statistics, Version 29.0.0.0 (241). Categorical variables were analyzed using Chi-square test or Fischer's exact test with Post hoc analysis with a Bonferroni correction. Baseline non-categorical variables were analysed using t-test or Mann-Whitney test after testing for

normality. For values of $P<0.05$, estimates were considered statistically significant. Univariate and multivariate Cox Regression analysis was performed to investigate factors affecting survival. Survival plots were generated with the Kaplan-Meier method. Log rank test was used to compare survival between groups.

Outcomes

Patient and tumor characteristics. Out of a total of 420 patients eligible for the study, 57(13.6%) cases were TNBC and 363(86.4%) non-TNBC. Observed frequencies and percentages for each cancer type are presented in Table I. Germline BRCA1 mutation was encountered in 3/57 cases of TNBC. The patients in the TNBC group accounted for 13.6% and 86.4% in the non-TNB gropup. The included patients were followed up for 1-96 months. The mean age at diagnosis for TNBC patients was lower (57.14 ± 12.62) than non-TNBC patients (60.53 ± 11.46), mean ± standard deviation. Median age for TNBC (59) and non-TNBC (62) was not statistically significantly different, U=11769, z=1.661, p=.097. The number of patients prior to menopause in the TNBC group accounted for 28.1%(16) and for 19.6%(71) in the non-TNBC. The two multinominal probability distributions for clinical TNM stage and pathological type were equal between groups, X^2 (2) = 0.926, p=.629 respectively Fischer's exact test p=.083. The most frequent encountered ICB-NST (84.2% TNBC vs. 84% non-TNBC) followed by lobular (7% vs. 11.6%) and metaplastic (5.3% vs. 0.8%) types. The distribution regarding tumor size in cm was not equal in the sample, $X^2(2) = 7.657$, p=.022. The chi-square test of homogeneity for tumor size showed that probability distributions were not equal in the groups $X^2(2) = 6.181$, p=.045. Post hoc analysis with a Bonferroni correction which a accepted statistical significance at p<.0166, resulted that all pairwise comparisons were not statistically significant. Tumor grade distributions were not equal, $X^2(2) = 79.441$, p=<.001. Statistical significance after Bonferroni correction was accepted at p<.0166. There were statistically significant differences in the proportion of grade 1 non-TNBC than TNBC (n=112, 30.9% versus n=4, 7%), as well as the proportion of grade 3 TNBC than non-TNBC (n=25, 43.9% versus n=20, 5.5%), p<.0166. There was no statistically significant differences in the proportions of grade 2 tumors TNBC than non-TNBC (n=28, 49.1% versus 231, 63.6%), p>.0166. A non-statistically significant difference in proportion between groups was also observed by

analysing nodal invasion, lymphovascular invasion (LVI), perineural invasion (PNI) and extra-nodal invasion (ENE), (Table I).

Table I. Clinicopathological characteristics of the patients breast cancer patients and comparison between tumor subgroups.

Characteristics	Total (n=420)	Subgroup, n(%) TNBC, 57(13.6)	Non-TNBC, 363(86.4)	P-value
Mean age at diagnosis		57.14	60.53	.097
Menopausal status (%)				.140
Prior to menopause	87	16(28.1)	71(19.6)	
Following menopause	333	41(71.9)	292(80.4)	
Clinical TNM stage				.629
I	47	5(8.8)	42(11.6)	
II	218	28(49.1)	190(52.3)	
III-IV	155	24(42.1)	131(36.1)	
Pathological type				.083
Invasive ductal carcinoma	353	48(84.2)	305(84.0)	
Invasive lobular carcinoma	46	4(7.0)	42(11.6)	
Metaplastic	6	3(5.3)	3(0.8)	
Other	15	2(3.5)	13(3.6)	
Tumor size (cm)				.045*,NS
≤2	147	24(42.1)	123(33.9)	
2-5.	228	23(40.4)	205(56.5)	
>5	45	10(17.5)	35(9.6)	
Tumor grade				<.001
I	116	4(7)	112(30.9)	
II	259	28(49.1)	231(63.6)	
III	45	25(43.9)	20(5.5)	
Nodal invasion				.668
Positive	254	33(57.9)	221(60.9%)	
Negative	166	24(42.1)	142(39.1)	
Invaded lymph nodes				.581
0	164	23(40.4)	141(38.8)	
1-3	153	24(42.1)	129(35.5)	
4-9	55	5(8.8)	50(13.8)	

>10	48	5(8.8)	43(11.8)	
Lymphovascular invasion				.627
Positive	76	9(15.8)	67(18.5)	
Negative	344	48(84.2)	296(81.5)	
Perineural invasion				.110
Positive	110	10(17.5)	100(27.5)	
Negative	310	47(82.5)	263(72.5)	
Extra-nodal extension				.976
Positive	154	21(36.8)	133(36.6)	
Negative	266	36(63.2)	230(63.4)	

*Bonferroni correction; NS, not statistically significant; TNBC, triple negative breast cancer

Recurrence. Within a mean follow-up period of 19.34 months (1-96 months), a DFS was observed in 314(74.8%) cases, whereas 106(25.2%) developed local recurrence or distant metastasis. There were no statistically significant differences in the proportion of non-TNBC that registered recurrence than TNBC (n=86, 23.7% versus n=20, 35.1%), or in the proportion of non-TNBC that had no recurrence than TNBC (n=277, 76.3% versus n=37, 64.9%) p.066. In the TNBC group 20 patients (35.1%) patients registered an event compared to 86 patients (23.7%) in the non-TNBC group, a non-statistically significant difference in proportions of .114, p=0.066. For TNBC group single metastatic site was encounterd in 7(35%) cases and multiple sites in 13(65%) cases whereas non-TNBC group registered 33(38.4%) patients with single metastatic site and 53(61.6%) with multiple sites, p.779. Frequently observed distant metastatic sites were the bone (29.3%), lung (24.5%), liver (17.8%), mediastinal lymph nodes (13.5%) and brain (7.2%), p.211. Associated data are presented in Table II. Mean DFS in the TNBC versus non-TNBC was 25.07 versus 21.88 months (U = 9201, z= -1.345, p = .179) and mean OS was 32.37 versus 26.34 months (U = 8806, z = -1809, p = .070), (Table II). Among the occurred tumor relapses 19 patients died, 5(8.8%) cases in TNBC group as compared to 14 cases (3.9%) in non-TNBC, [$X^2(1)=2.756$, p.158].

Table II. Metastatic status and sites of recurrence.

	Subgroup,

	Total (n,%)	TNBC n(%)	Non-TNBC n(%)	
Recurrence or metastasis				.066
Yes	106(25.2)	20(35.1)	86(23.7)	
No	314(74.8)	37(64.9)	227(76.3)	
Metastatic site				
Brain	15(7.2)	5(12.8)	10(5.9)	.133
Bone	61(29.3)	8(20.5)	53(41.4)	.180
Liver	37(17.8)	5(12.8)	32(18.9)	.368
Lung	51(24.5)	9(23.1)	42(24.9)	.816
Mediastinal lymph nodes	28(13.5)	8(20.5)	20(11.8)	.152
Pleura	4(1.9)	0(0)	4(2.4)	.433
Splenic	2(1)	0(0)	2(1.2)	.659
Omental/Peritoneal	1(0.5)	0(0)	1(0.6)	.812
Ovarian	2(1)	1(0.6)	1(2.6)	.341
Locoregional	7(3.4)	3(7.7)	4(2.4)	.124

TNBC, triple negative breast cancer.

Survival analysis. Pathological type [$X^2(3)=27.596$, p<.001], recurrence (no vs. yes) [$X^2(1)=339.588$, p<.001], tumor size [$X^2(2)=58.668$, p<.001], tumor grade [$X^2(2)=9.507$, p.009], axillary lymph node status [$X^2(1)=43.160$, p<.001], lymph node metastasis [$X^2(2)=50.419$, p<.001], LVI [$X^3(1)=40.525$, p<.001], PNI [$X^2(1)=6.317$, p.012], ENE [$X^2(1)=60.815$, p<.001] and recurrence status (positive vs. negative) [$X^2(1)=339.588$, p<.001] emerged as significant prognostic factors for RFS in univariate analysis. Comparing the pathological type of the tumors, regression coefficients for lobular (B=.533, SE=.244, p=.029) and metaplastic type (B=2.088, SE=.397, p=<.001) were statistically significat positive, whilst the group „other" was negatively predictive of the hazard for relapse with a negative coefficient of -11.590 (SE=176.362, p=.948) and showed no replase in the observational period.

Age had a non-statistically significance but a positive regression coefficient of .239 (SE=242, p=.323) [$X^2(1)=1.025$, p.311], which indicated that patients with age above 49 were positive associated with relapse earlier than patients with age ≤49. A positive coefficient of .202 (SE=234, p=.388) [$X^2(1)=.776$, p.378] for patients after menopause indicated a positive assosiation with relapse. TNBC was not significat different compared to non-TNBC in terms of RFS but a positive regression coefficient of .304

(SE=249, p=.222) [$X^2(1)=1.397$, p.237] indicated a positive relationship to recurrence, (Table III).

In the univariate analysis for OS, significant prognostic factors were pathological type [$X^2(3)=10.671$, p.014] (metaplastic type B=2.671, SE=.657, p<.001, lobular type B=.336, SE=.642, p.600), tumor size [$X^2(2)=7.915$, p=.019] (>5 cm, B=1.903, SE=.708, p=.007), positivity vs. negativity of invaded lymph nodes [$X^2(1)=7.038$, p.008] (positive nodes, B=1.626, SE=.749, p<.030), pathological nodal stage [$X^2(2)=14.355$, p<.001], LVI [$X^2(1)=13.056$, p<.001], ENE [$X^2(1)=20.286$, p<.001] and recurrence status [$X^2(1)=39.618$, p<.001]. Predictive positive regression coefficients with a non-statistically significance as age >49 [$X^2(1)=.024$, p.876] (B=0.087, SE=.563, p.877), TNBC compared to non-TNBC [$X^2(1)=1.103$, p.294] (B=575, SE=.522, p.270) and positive PNI [$X^2(1)=1.215$, p.270] (B=.572, SE=.499, p.252) indicated nevertheless a positive relationship between the covariates and the hazard for the terminal event. Group following menopause had a negative coefficient of -.082 (SE=.522, p.875) [$X^2(1)=.025$, p.876] in relationship with the hazard, assessing menopause as protective factor to event in the OS analysis and predicted patients prior to menopause to suffer an event earlier than those following menopause, (Table III).

Table III. Univariate analysis of factors related to disease free survival and overall survival.

	Disease free survival			Overall survival		
Characteristics	HR	95% CI	P-value	HR	95% CI	P-value
Age (≤49 vs. >49)	1.270	0.790 - 2.042	.323	1.091	0.362 - 3.292	.877
Menopausal status (prior to vs. after)	1.224	0.774 - 1.937	.388	0.921	0.331-2.562	.875
Tumor subgroups (TNBC vs. non-TNBC)	1.355	0.832 - 2.207	.222	1.778	0.639 - 4.944	.270
Pathological type						
NST vs. Lobular	1.704	1.056 - 2.751	.029	1.400	0.398 - 4.922	.600
NST vs. Metaplastic	8.069	3.703 - 17.582	<.001	14.456	3.990 - 52.382	<.001
NST vs. Other	0.000	0.000 - 1.220	.948	0.000	0.000	.980
Tumor size						
≤2 vs. 3-5 cm	2.369	1.386 - 4.049	.002	1.908	0.524 - 6.944	.327

≤2 vs. >5cm	9.110	5.077 - 16.349	<.001	6.706	1.675 - 26.844	.007
Tumor Grade						
I vs. II	1.636	1.006 - 2.660	.047	3.258	0.738 - 14.372	.119
I vs. III	2.616	1.416 - 4.831	.002	3.193	0.533 - 19.119	.204
Axillary LN status (negative vs. positive)	4.691	2.718 -8.097	<.001	5.082	1.172 - 22.047	.030
Lymph node metastasis						
≤3 vs. 4-9	2.904	1.834 - 4.600	<.001	4.043	1.231 - 13.283	.021
≤3 vs. ≥10	5.078	3.242 - 7.953	<.001	7.244	2.512 - 20.887	<.001
LVI (negative vs. positive)	3.790	2.583 - 5.563	<.001	5.767	2.308 -14.407	<.001
PNI (negative vs. positive)	1.678	1.135 - 2.480	.009	1.771	0.666 - 4.711	.252
ENE (negative vs. positive)	4.563	3.063 - 6.798	<.001	10.281	2.995 - 35.298	<.001
Recurrence (no vs. yes)	427.640	59.69 -3063.55	<.001	15.395	2.581-9183.06	.016
Metastatic site (single vs. multiple)	0.888	0.610 - 1.293	.535	3.850	0.884 - 16.761	.072

TNBC, triple negative breast cancer; LN, lymph node; LVI, lymphovascular invasion; PNI, perineural invasion; ENE, extra-nodal extension.

Significant prognostic factors in univariate analysis were included in the multifactor Cox proportional hazard regression model (Tabel IV). The result of the test sugessted that the model for DFS was statistically significant [$X^2(8)=111.454$, $p<.001$]. Pathological type, tumor size, tumor grade, nodal stage, LVI and ENE were independent prognostic factors for recurrence with statistical significance. Multivariate analysis regarding OS was also statistically significant, [$X^2(7)=30.091$, $p<.001$], however ENE was the only statistically significat independent prognostic factor.

Table IV. Multivariate analysis of factors related to disease free survival and overall survival.

	Disease free survival			Overall survival		
Characteristics	HR	95% CI	P-value	HR	95% CI	P-value
Pathological type (NST vs. others)	1.690	1.082 -2.639	.021	1.416	0.513 - 3.907	.502

Tumor size (≤2 vs. >2 cm)	1.976	1.135-3.441	.016	1.249	0.309 - 5.051	.755
Tumor Grade (I-II vs. III)	1.633	1.002 -2.659	.049	1.324	0.368 - 4.760	.668
Axillary NS status (negative vs. positive)	1.462	0.741 -2.882	.273	0.503	0.065 - 3.916	.512
Lymph node metastasis ≤3 vs. ≥ 4	1.774	1.154 -2.729	.009	2.515	0.781 - 8.101	.122
LVI (negative vs. positive)	1.943	1.237 -3.052	.004	2.444	0.844 - 7.075	.099
PNI (negative vs. positive)	0.817	0.524 -1.272	.371		not included	
ENE (negative vs. positive)	2.750	1.686 -4.486	<.001	7.307	1.453-36.756	.016

TNBC, triple negative breast cancer; LN, lymph node; LVI, lymphovascular invasion; PNI, perineural invasion; ENE, extra-nodal extension.

The Kaplan-Meier survival curves are shown in Fig.1. Patients in the non-TNBC group had a mean time to relapse of 72.0 (95% CI, 55.81 to 88.18) months. This was longer than the TNBC group, with a mean time to relapse of 60.0 (95% CI, 37.16 to 82.83) months. The percentage of censored cases present in the non-TNBC (76.6%) and TNBC (64.9%) groups was similar. A log rank test was run to determine if there were differences in the relapse distributions for the different types of BC. The relapse distributions for the two groups were not statistically significantly different, $X^2(1)=1.524$, p.217. Regarding OS, patients in the non-TNBC had a mean time to event of 80.53 months (95% CI, 77.75 to 83.32), identical to mean time to event of TNBC of 80.04 month (95% CI, 66.76 to 93.33). Log rank test had no statistically significantly different survival distributions, $X^2(1)=1.252$, p.263.

Figure 1a. Kaplan-Meier curve for disease free survival.

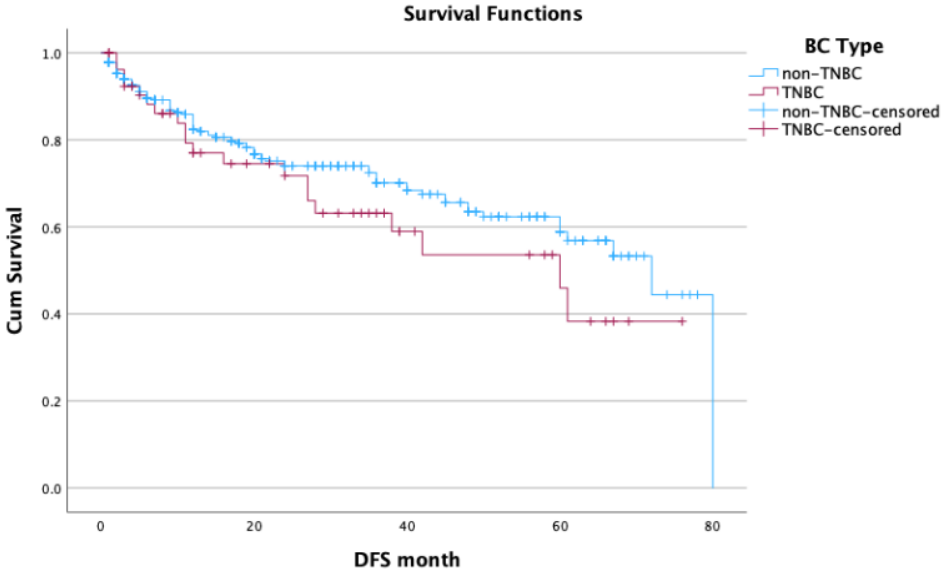
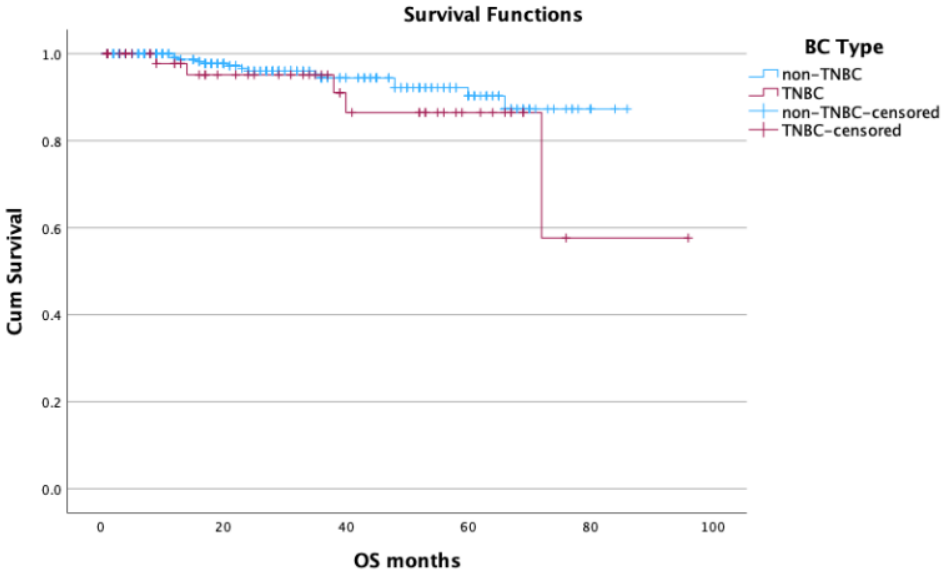


Figure 1b. Kaplan-Meier curve for overall survival



Discussion

In many studies TNBC is associated with younger age [9-11]. Many studies have demonstrated that premenopausal African-American females were more prone to develop TNBC [12-14]. The current study demonstrated that age and menopausal

status did not significantly affect the incidence in our groups, mean age was 57.14 (95% CI 53.7 to 60.49) and only 28.1% patients were prior to menopause.

Triple negative breast cancer had demonstrated an agressive nature [15]. Clinical trials reported larger tumor size, high-grade tumors and advanced nodal stage [16]. Kendal et al reported an average tumor size of 2 cm [17]. TNBC are described as most often high grade invasive ductal carcinomas, although there are some rare histological subtypes such as adenoid cystic carcinoma of the breast that is associated with an excellent prognosis [18]. In our study 42.1% had tumor size ≤2cm, presented higher tumor grade, respectively grade II (49.1% and grade III (43.9), 57.9% were positive for nodal invasion and 42.1% had between 1 and 3 invaded nodes. Lobular type (7%) and metaplastic type (5.3%) were the most often patological type encountered after IBC-NST (84.2%) in TNBC.

In literature TNBC is commonly associated with increased risk of visceral metastasis including lung, liver and brain as compared to bone metastases [19,20]. In our group brain metastasis had only 7.2%, most frequent site was bone (29.3%) followed by lung (24.5%) and liver (17.8%).

Results in the univariate Cox regression analysis have exhibited that tumor type, tumor size, tumor grade, lymph node metastasis, LVI, PNI and extra-nodal extension were prognostic indicators for DFS and OS. These findings are in agreement with other studies showing that advanced clinical stage, larger tumor size, angiolymphatic invasion and positive nodal involvement were related to increased recurrence and reduction in OS [21-24]. Keiko et al reported that PNI-posive patients had short distant metastases-free survival, that the disease free survival status was significantly correlated with large pathological tumor size, lymph node metastases and lymphatic invasion and that according to the multivariate analysis, PNI was an independent factor of poor prognostic [25,26]. In the current study PNI positive status had a statistically significant coefficient for DFS and a positve non-significant coefficient for OS. Extracapsular extension of nodal metastases is a frequent histologic finding that was prognostically significant in previous many reports [27,28]. In the multivariante analysis extra-nodal extension was strongly related with a poor prognostic both DFS and OS. Studies in literature have reported TNBC as an independent prognostic factor and controversaly, some studies have not shown TNBC as a poor prognostic factor [9,11,30-32]. In our study although showed a non-

statistically significance, TNBC showed a lower time to relapse compared to non-TNBC.

Conclusion

37

This study has limitations regarding the sample size, further the patients who had not molecular diagnostic available were excluded, the mean follow-up is also less and futher the percentage of loss to follow-up is high. Nevertheless TNBC subtypes have demonstrated a large homogeneity in terms of tumor developement and distinct prognoses as a consequence development of targeted therapies of the distinct molecular profiles is required.

References

1. Metzger-Filho O, Tutt A, de Azambuja E, Saini KS, Viale G, Loi S, et al. Dissecting the heterogeneity of triple-negative breast cancer. *J Clin Oncol*. 2012 May 20;30(15):1879-87. doi: 10.1200/JCO.2011.38.2010. Epub 2012 Mar 26. PMID: 22454417.

2. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer*. 2007 Jan 1;109(1):25-32. doi: 10.1002/cncr.22381. PMID: 17146782.

3. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011 Jul;121(7):2750-67. doi: 10.1172/JCI45014. PMID: 21633166; PMCID: PMC3127435.

4. Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y et al.. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One*. 2016 Jun 16;11(6):e0157368. doi: 10.1371/journal.pone.0157368. PMID: 27310713; PMCID: PMC4911051.

5. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SA, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. *Clin Cancer Res*. 2015 Apr 1;21(7):1688-98. doi: 10.1158/1078-0432.CCR-14-0432. Epub 2014 Sep 10. PMID: 25208879; PMCID: PMC4362882.

6. Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol*. 2006 Feb;19(2):264-71. doi: 10.1038/modpathol.3800528. PMID: 16341146.
7. Cakir A, Gonul II, Uluoglu O. A comprehensive morphological study for basal-like breast carcinomas with comparison to nonbasal-like carcinomas. *Diagn Pathol*. 2012 Oct 20;7:145. doi: 10.1186/1746-1596-7-145. PMID: 23082819; PMCID: PMC3488514.
8. Lakhani SR, Jacquemier J, Sloane JP, Gusterson BA, Anderson TJ, van de Vijver MJ et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst*. 1998 Aug 5;90(15):1138-45. doi: 10.1093/jnci/90.15.1138. PMID: 9701363.
9. Gogia A, Raina V, Deo SV, Shukla NK, Mohanti BK. Triple-negative breast cancer: An institutional analysis. *Indian J Cancer*. 2014 Apr-Jun;51(2):163-6. doi: 10.4103/0019-509X.138275. PMID: 25104201.
10. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-Negative Breast Cancer: Clinical Features and Patterns of Recurrence. *Clin Cancer Res*. 2007;13:4429–34
11. Haffty BG, Yang Q, Reiss M, Kearney T, Higgins SA, Weidhaas J, et al Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. *J Clin Oncol*. 2006;24:5652–7
12. Yuan N, Meng M, Liu C, Feng L, Hou L, Ning Q et al. Clinical characteristics and prognostic analysis of triple-negative breast cancer patients. *Mol Clin Oncol*. 2014 Mar;2(2):245-251. doi: 10.3892/mco.2013.230. Epub 2013 Dec 20. PMID: 24649341; PMCID: PMC3917786.
13. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer*. 2007 Jan 1;109(1):25-32. doi: 10.1002/cncr.22381. PMID: 17146782.
14. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*. 2007 Aug 1;13(15 Pt 1):4429-34. doi: 10.1158/1078-0432.CCR-06-3045. PMID: 17671126.
15. Sharma D, Singh G. An institutional analysis of clinicopathological features of triple negative breast cancer. *Indian J Cancer*. 2016 Oct-Dec;53(4):566-568. doi: 10.4103/ijc.IJC_534_16. PMID: 28485352.

16. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med*. 2010 Nov 11;363(20):1938-48. doi: 10.1056/NEJMra1001389. PMID: 21067385.
17. Kandel MJ, Stadler Z, Masciari S, et al. Prevalence of BRCA1 mutations in triple negative breast cancer (BC). *J Clin Oncol*. 2006;24:508.
18. O'Reilly D, Sendi MA, Kelly CM. Overview of recent advances in metastatic triple negative breast cancer. *World J Clin Oncol*. 2021 Mar 24;12(3):164-182. doi: 10.5306/wjco.v12.i3.164. PMID: 33767972; PMCID: PMC7968109.
19. Jin J, Gao Y, Zhang J, Wang L, Wang B, Cao J et al. Incidence, pattern and prognosis of brain metastases in patients with metastatic triple negative breast cancer. *BMC Cancer*. 2018 Apr 19;18(1):446. doi: 10.1186/s12885-018-4371-0. PMID: 29673325; PMCID: PMC5909254.
20. Lin NU, Claus E, Sohl J, Razzak AR, Arnaout A, Winer EP. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. *Cancer*. 2008 Nov 15;113(10):2638-45. doi: 10.1002/cncr.23930. PMID: 18833576; PMCID: PMC2835546.
21. Costa READRD, Oliveira FTR, Araújo ALN, Vieira SC. Prognostic factors in triple-negative breast cancer: a retrospective cohort. *Rev Assoc Med Bras* (1992). 2021 Jul;67(7):950-957. doi: 10.1590/1806-9282.20210249. PMID: 34817505.
22. Ovcaricek T, Frkovic SG, Matos E, Mozina B, Borstnar S. Triple negative breast cancer - prognostic factors and survival. *Radiol Oncol*. 2011 Mar;45(1):46-52. doi: 10.2478/v10019-010-0054-4. Epub 2010 Dec 31. PMID: 22933934; PMCID: PMC3423721.
23. Mousavi SA, Kasaeian A, Pourkasmaee M, Ghavamzadeh A, Alimoghaddam K, Vaezi M et al. Assessing the prognostic factors, survival, and recurrence incidence of triple negative breast cancer patients, a single center study in Iran. *PLoS One*. 2019 Jan 4;14(1):e0208701. doi: 10.1371/journal.pone.0208701. PMID: 30608935; PMCID: PMC6319722.
24. Ahn KJ, Park J, Choi Y. Lymphovascular invasion as a negative prognostic factor for triple-negative breast cancer after surgery. *Radiat Oncol J*. 2017 Dec;35(4):332-339. doi: 10.3857/roj.2017.00416. Epub 2017 Dec 15. PMID: 29232804; PMCID: PMC5769883.

25. Hosoya K, Wakahara M, Ikeda K, Umekita Y. Perineural Invasion Predicts Unfavorable Prognosis in Patients With Invasive Breast Cancer. *Cancer Diagn Progn*. 2023 Mar 3;3(2):208-214. doi: 10.21873/cdp.10203. PMID: 36875309; PMCID: PMC9949536.
26. Yekedüz E, Dizdar Ö, Kertmen N, Aksoy S. Comparison of Clinical and Pathological Factors Affecting Early and Late Recurrences in Patients with Operable Breast Cancer. *J Clin Med*. 2022 Apr 22;11(9):2332. doi: 10.3390/jcm11092332. PMID: 35566457; PMCID: PMC9105518.
27. Donegan, W.L., Stine, S.B. and Samter, T.G. (1993), Implications of extracapsular nodal metastases for treatment and prognosis of breast cancer. *Cancer*, 72: 778-782. https://doi.org/10.1002/1097-0142(19930801)72:3<778::AID-CNCR2820720324>3.0.CO;2-J
28. J.A. Bucci, C.W. Kennedy, J. Burn, D.J. Gillett, H.L. Carmalt, M.J. Donnellan et al. Implications of extranodal spread in node positive breast cancer: a review of survival and local recurrence. *The Breast*. 2001;10(3):213-219. ISSN 0960-9776, https://doi.org/10.1054/brst.2000.0233.
29. A. Nottegar, N. Veronese, M. Senthil, R.M. Roumen, B. Stubbs, A.H. Choi et al. Extra-nodal extension of sentinel lymph node metastasis is a marker of poor prognosis in breast cancer patients: A systematic review and an exploratory meta-analysis. *EJSO*. 2016;42(7):919-925. ISSN 0748-7983, https://doi.org/10.1016/j.ejso.2016.02.259.
30. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res*. 2007 Apr 15;13(8):2329-34. doi: 10.1158/1078-0432.CCR-06-1109. PMID: 17438091.
31. Kaplan HG, Malmgren JA. Impact of triple negative phenotype on breast cancer prognosis. *Breast J*. 2008 Sep-Oct;14(5):456-63. doi: 10.1111/j.1524-4741.2008.00622.x. Epub 2008 Jul 24. PMID: 18657139.
32. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer*. 2007 May 1;109(9):1721-8. doi: 10.1002/cncr.22618. PMID: 17387718.